JOURNAL OF MICROBIOLOGY & BIOLOGY EDUCATION, December 2016, p. 490-491
DOI: http://dx.doi.org/10.1128/jmbe.v17i3.1180

Exploring Catalase and Invertase Activity Using Sodium Alginate-Encapsulated Yeast (Yeast Spheres) †

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INTRODUCTION

Finding the right enzyme experiment can be problematic, depending what one is trying to show, what supplies and equipment are available, and the time one can devote to the topic. Experiments range from those that only indicate presence/absence of enzyme activity (4) to those that require time-consuming isolation procedures and spectrophotometric analysis (1, 2, 6). I have developed simple and inexpensive procedures for looking at catalase and invertase activity using yeast encapsulated in sodium alginate, a technique that has been used for several applications, including the production of alcoholic beverages (3) and for investigating fermentation in teaching labs (5).

Catalase activity using yeast spheres may be explored with or without gas pressure sensors. Without sensors available, an easy and very reliable method is to use graduated cylinders filled with H_2O_2. A yeast sphere is dropped into a graduated cylinder containing H_2O_2, the substrate. The sphere will sink to the bottom of the cylinder and then, as the catalase produced by the yeast reacts with the hydrogen peroxide to form O_2 gas bubbles around the sphere, the sphere will rise to the surface. Because the reaction is quick, multiple replicates may be performed in a very short period of time, and thus statistics can easily be incorporated into the data analysis. With sensors, a few grams of yeast spheres are added to an Erlenmeyer flask containing H_2O_2, the sensor is attached, and pressure readings are recorded. Experiments may be designed to test the effect of substrate (H_2O_2) concentration, temperature, or pH on the catalase reaction. The trends in activity are similar no matter which method is used (Appendix 1).

Invertase catalyzes the hydrolysis of sucrose to glucose and fructose; thus the activity of invertase may be measured by the appearance of glucose. With yeast cells encapsulated in sodium alginate, there are no cells in solution to interfere with the various ways to test for glucose. One of the easiest ways to test for glucose is to use urine analysis test strips. The strips are dipped into the solution surrounding the spheres and the color change is compared to a color chart: the more glucose in solution the darker the color. If a more quantitative method is desired, samples from the solution surrounding the yeast spheres may be withdrawn and tested for the presence of glucose using glucose oxidase. Experiments may be designed to test the effect of temperature or substrate (sucrose) concentration on the invertase reaction. The results mirror each other no matter what method is used (Appendix 1).

PROCEDURE

Preparation of yeast spheres

A day or two before the lab period, make a 2% sodium alginate solution in beakers or plastic cups, and leave it out at room temperature. The sodium alginate solution is viscous and takes a while to get into solution which is why I recommend making a separate beaker/cup for each lab group instead of making a big batch and trying to aliquot it out when ready to use (Appendix 2).

On the day of the experiment, about 10 minutes before students will be making their yeast spheres, make a 10% solution of yeast in warm tap water (I use Fleischman's RapidRise bread yeast, *Saccharomyces cerevisiae*). Have students add an equal volume of the yeast suspension to their cup of 2% sodium alginate. This solution should be mixed well and then drawn up into a syringe. A plastic pipet may also be used, but syringes give a little more control and consistency in size and also allow more yeast spheres to be made at once.

All excess liquid should be wiped off the syringe, and the syringe then held over a cup or beaker containing 0.15M $CaCl_2$. As the syringe plunger is very slowly depressed, uniform drops of the yeast-sodium alginate solution will form spheres as they come in contact with the $CaCl_2$ solution and these will fall to the bottom of the beaker. After all of the yeast-sodium alginate is dispensed, any floating spheres should be discarded and the rest of the spheres rinsed in tap water and drained (Fig. 1). At this point, the spheres are ready to be tested for enzyme activity. If time

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FIGURE 1. Yeast spheres.

is short, the spheres may be placed in tap water and put into a refrigerator until ready to be used.

Designing an experiment

Catalase. For the initial trial runs using the graduate method, students should fill a graduated cylinder with 0.3% H_2O_2. I generally use 50 mL cylinders with 5 mL 3% H_2O_2 (the concentration of store-bought H_2O_2) + 45 mL dH_2O. Using forceps, or the loop end of the inoculating loop, one yeast sphere is dropped into the graduated cylinder. Students should start timing as soon as the sphere touches the bottom of the cylinder. Timing continues until the sphere reaches the surface. The used yeast sphere is disposed of and the experiment is repeated with a few more spheres to get several time samples. Students should use spheres that are close in size for all of their trials. For gas pressure sensor experiments, I use 5 g of yeast spheres in 50 mL of 1.5% H_2O_2 and run the program for two minutes.

Once the basics of the set-up are understood, students are free to design an experiment to see what effect different variables will have on this enzymatic reaction. Make sure they write out their experimental design first before carrying out the experiment, including the control, what concentration(s) of substrate will be used, what temperature(s), how many trials, and how the data will be analyzed and displayed.

Invertase. For initial runs for either the test stick or glucose oxidase method, I use one gram of yeast spheres in 10 mL of a 2.5% sucrose solution. At different time periods, glucose may be tested for by either dipping glucose test strips into the surrounding solution or taking out 0.2 mL of the solution and adding it to 1.8 mL of dH_2O and 1 mL of glucose oxidase (Appendix 2).

CONCLUSION

These are very easy and reliable enzyme experiments that enable students to collect data in a relatively short period of time. The graduated cylinder method for testing catalase is especially good for collecting large amounts of data that enable students to use statistics and, unlike similar yeast catalase experiments using paper disks and a yeast solution (http://cibt.cornell.edu/labs-activities/labs/catalase/), the yeast spheres are easy to manipulate, and there is very little variability. I have used this procedure with students in class and with teachers in workshops, with positive results and comments (Appendix 3). The gas pressure sensor method works nicely as well but requires more yeast spheres and takes a bit more time. Testing for invertase activity is a nice addition to a respiration/fermentation lab, and when used in conjunction with catalase, gives a nice comparison as to how enzymes, even from the same organism, may have different temperature and pH optima.

SUPPLEMENTAL MATERIALS

Appendix 1: Sample data
Appendix 2: Preparation background and supplies
Appendix 3. Sample instructions for testing catalase activity

ACKNOWLEDGMENTS

The author declares that there are no conflicts of interest.

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